

Functional potential of microbial communities of the Western Sahara salt plains

Velislava I. Ilieva^{1*}, Ben P. Stephens¹, Tim Goodall², Daniel Read², Victoria K. Pearson¹, Karen Olsson-Francis¹ and Michael C. Macey¹

¹ AstrobiologyOU, The Open University, Milton Keynes, United Kingdom
² Centre for Hydrology and Ecology, Wallingford, United Kingdom
 *corresponding author

@ilievavelislava velislava.ilieva@open.ac.uk

Introduction

Early Mars is thought to have been more **habitable** than present-day Mars. Evidence from the analysis of martian meteorites, satellite data, and rover/lander missions data support the presence of **liquid water, atmosphere, potential energy sources (e.g., chemical compounds in different oxidation states such as sulfides and sulfates), and a magnetic field** on early Mars (Grotzinger et al., 2014). These features are key in sustaining life on Earth and protecting against radiation. Therefore, early Mars could have potentially supported **Earth-like life**.

During the **Noachian-Hesperian transition period**, 3.5 - 3.8 billions of years ago, Mars became less conducive to life: **weakened magnetic field, evaporation of surface water, concentration of salt-bearing mineral phases, thinning atmosphere, high intensity UV rays** that were able to reach the surface (Warner et al., 2010). If Earth-like life was present on Mars, how it would have fared under these new conditions?

Western Sahara salt plains can be used to study microbial communities that are adapted to **high salinity, low water availability, low nutrient availability, intense UV radiation**. These conditions are thought to have occurred on Mars following the Noachian-Hesperian transition period and can, therefore, teach us about possible life on Mars.

Aim: characterise the microbial diversity and functional potential of the Western Sahara salt plains and investigate the chemistry of the sites.

Methods

Field site:

- Western Sahara salt plains
- Site 1 - thick salt crust
 - Site 2 - wet sand/sediment
 - Site 3 - dry sand

Samples:

- Salt crystals
- Sediment
- Water

Chemical analysis:

- Ion chromatography
- Inductively-coupled plasma optical emission spectroscopy
- Scanning electron microscopy

Microbiological analysis:

- DNA extraction
- 16S rRNA gene amplicon and metagenomic sequencing
- Cultivation of sulfate-reducing bacteria



Taxonomic diversity (who is there?)

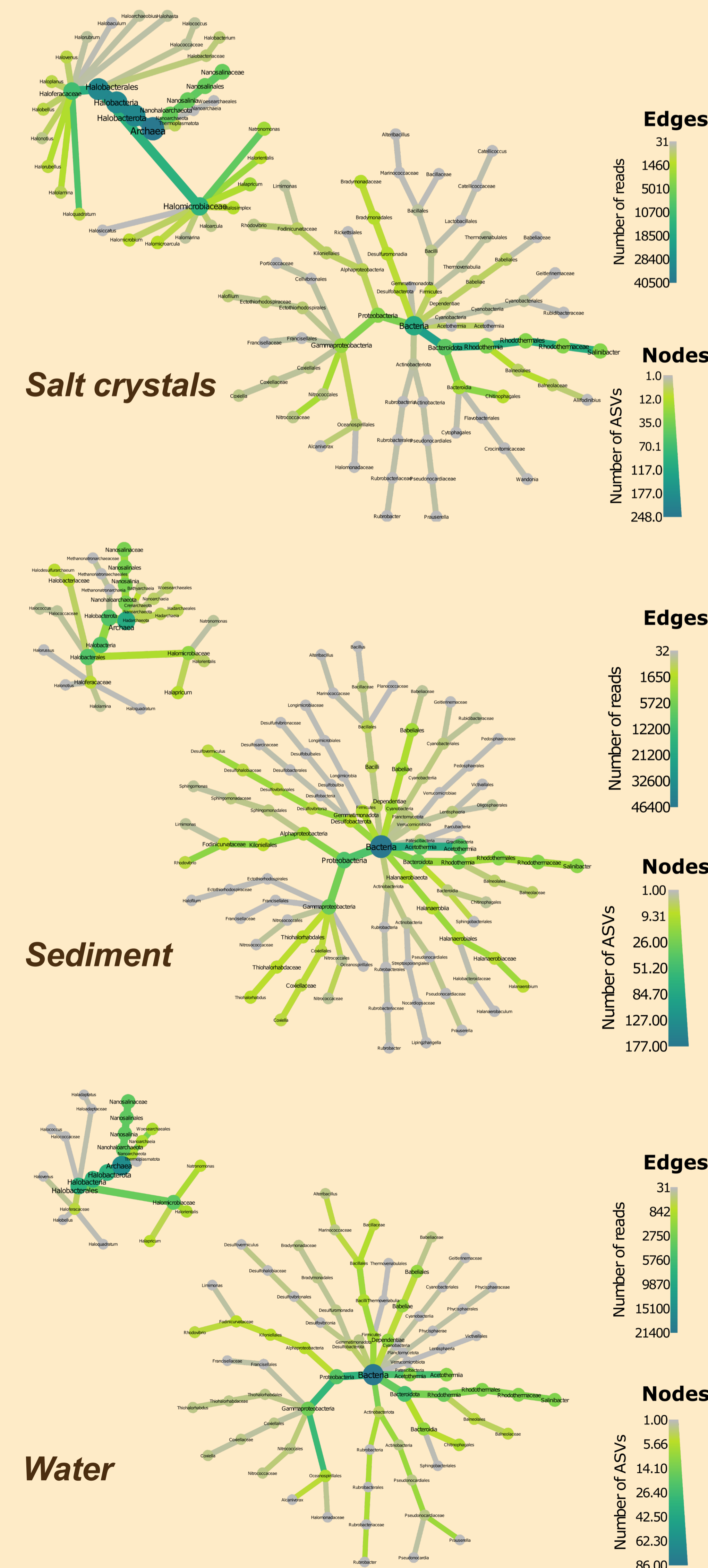


Fig.3. The taxonomic abundance of bacterial and archaeal 16S rRNA gene sequences. Heat trees were generated in R (4.2.1) with packages vegan (2.6-2) and metacoder (0.3.5). Amplicon sequence variants (ASVs) with less than 30 counts were removed from the data.

What is the chemistry of the environment?

Salt crystals

[Na] and [Cl] were above the detection limit, suggesting that **halite (NaCl salt)** is the dominant salt phase.

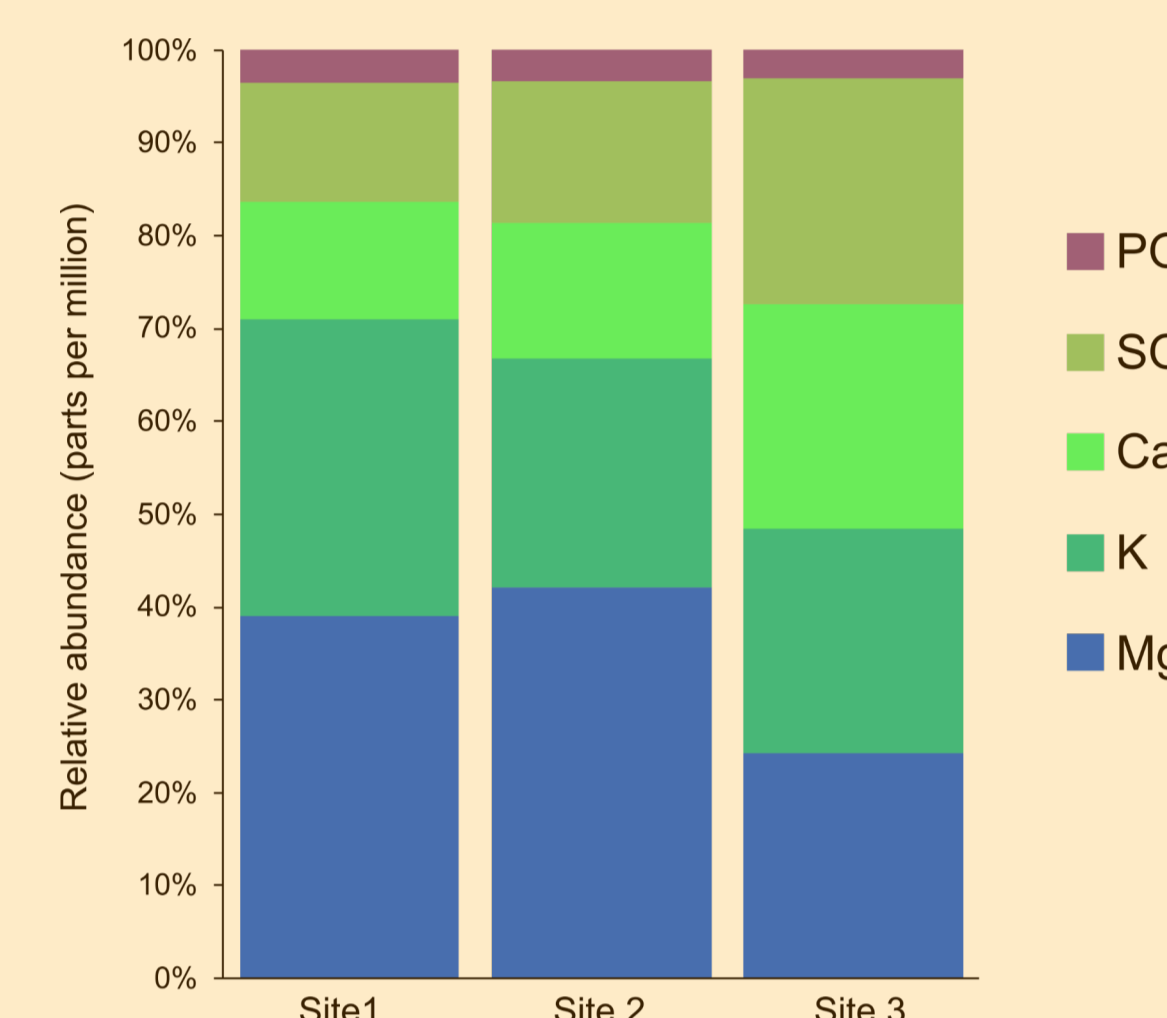


Fig. 1. Relative abundance of chemical elements and bio-essential anions.

Sediment

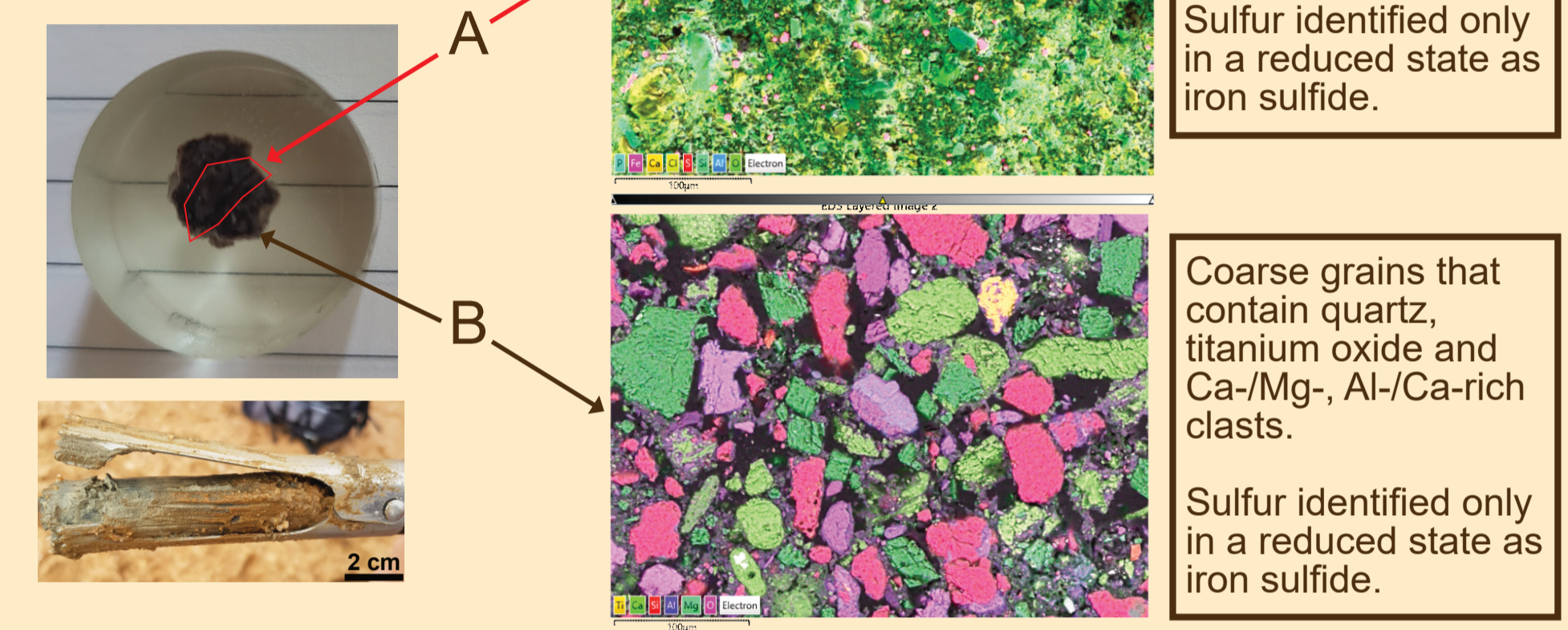


Fig. 2. Elemental maps of a sediment sample.

Functional potential (what are they doing?)

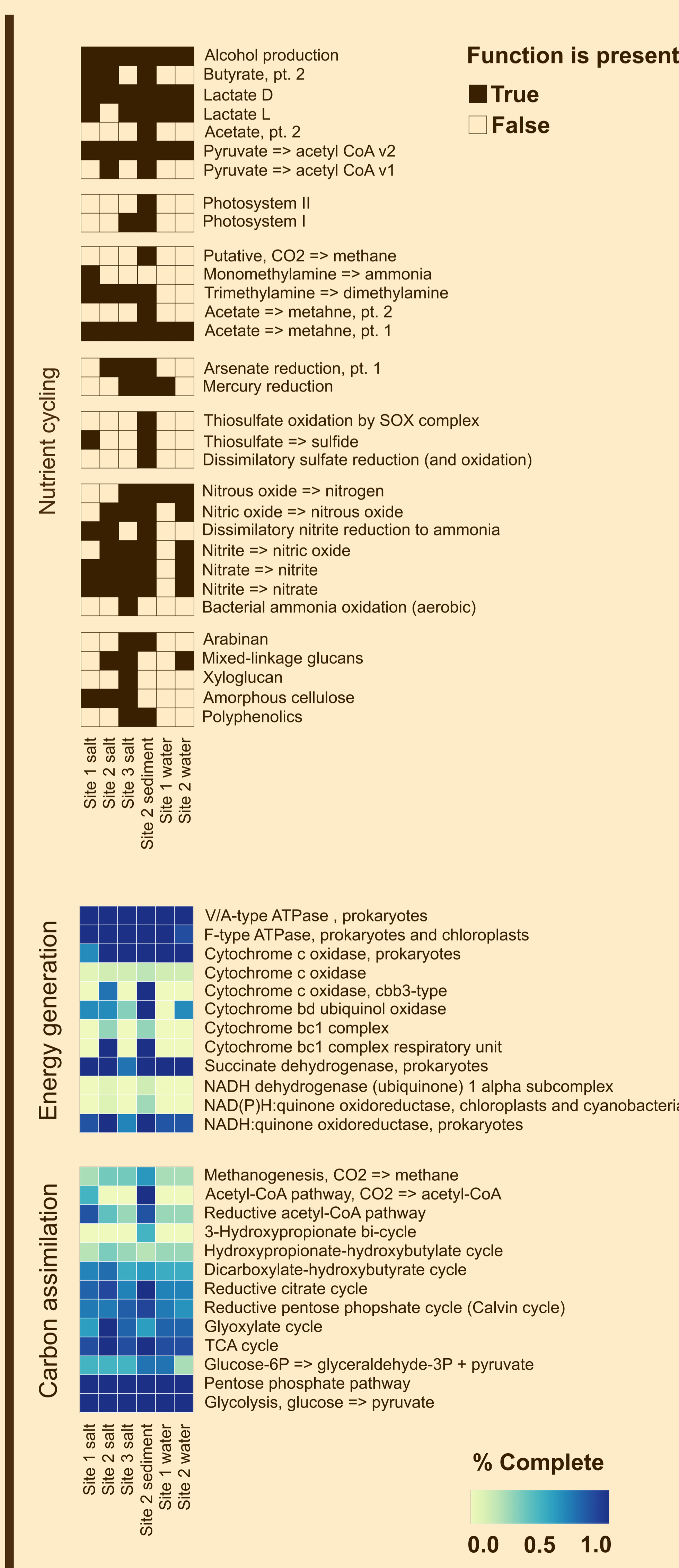


Fig.4. Metabolic pathways of salt, sediment and water metagenomes. Metagenome assemblies were annotated with dram (version 0.1.2). (Top) Presence/absence of specific functions. (Bottom) Percent completion of functional modules.

Cultivation (active microbes)

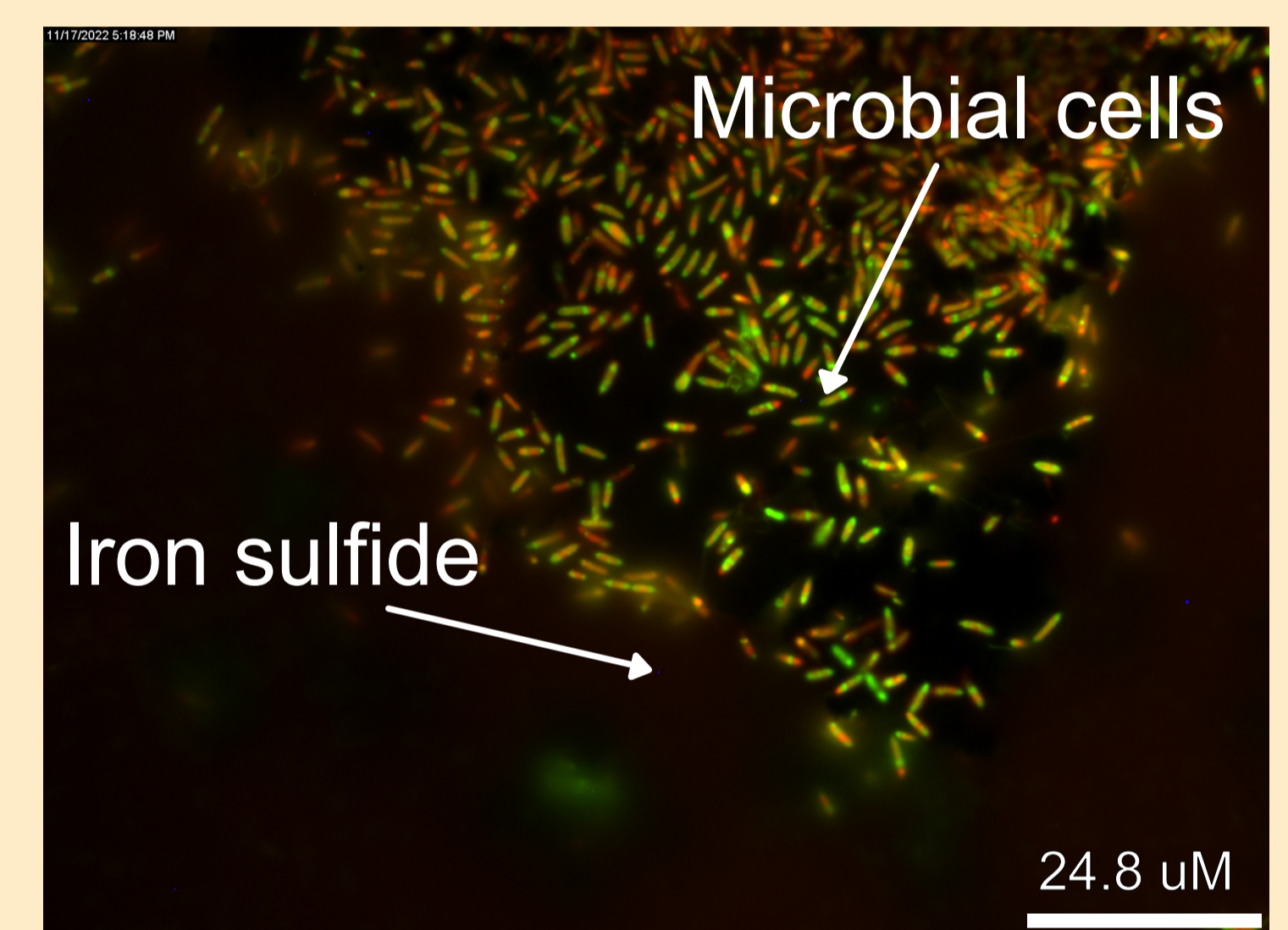


Fig.5. Live/dead staining of *Desulfosporosinus merdicii* grown in anaerobic conditions. Green cells are alive, red cells are dead, and orange cells are dying. Oxygen is toxic for *D. merdicii*, and we see here that many cells have stained orange because of oxygen exposure during microscopy.

Results and Conclusions

- Chemical analysis (Fig.1) showed that sodium and chloride are most abundant in the samples, suggesting that **halite is the dominant salt phase in the Western Sahara salt plains**. Scanning electron microscopy (Fig.2) revealed that clay-bearing phases are likely present in the sediments at Site 2.
- **The Western Sahara salt plains are a physico-chemical analogue for chloride-bearing deposits on Mars, even though the geological setting is different.**
- **Extremely halophilic Archaea and Bacteria dominated** the salt crystals, sediment and water samples (Fig.3). Archaeal sequences are more abundant in the harsh conditions of the surface salts, whereas bacterial sequences are more abundant in the sediment and water fractions.
- Functional profiling identified that the sediment hosts a diversity of energy-yielding metabolisms that thrive in the absence of oxygen (Fig.4).
- Sequencing identified that sulfur cycling microbes were present in the sediment and the genes that they require to obtain energy from sulfur (Figs.3 and 4). A sulfate-respiring microbe was **successfully cultured** in the lab (Fig.5).
- **Chemolithotrophic metabolisms (such as sulfur cyclers) are considered models for hypothetical life on Mars (Macey, et al., 2020).**

Future work

Future work will reconstruct genomes from the sequencing data to further analyse the resident microbes of the salt, sediment and water. Comparison study between Western Sahara salt plains and hypersaline arid lakes in La Mancha (Spain) will be carried out to identify how different chemistries influence the microbiology of high-salt environments and what is the effect of geography.

Grotzinger, J. P., Sumner, D. Y., Kah, L. C., Stack, K., Gupta, S., Edgar, L., Moore, J.M., et al. (2014). A Habitable Fluvio-Lacustrine Environment at Yellowknife Bay, Gale Crater, Mars. *Science*, 343(6159), 1242-1277. <https://doi.org/10.1126/science.1242777>. Warner, N., Gupta, S., Lin, S.-Y., Kim, J.-R., Muller, J.P., & Morley, J. (2010). Late Noachian to Hesperian climate change on Mars: Evidence of episodic warming from transient crater lakes. *Planetary and Space Science*, 58(12), 1145-1150. <https://doi.org/10.1016/j.pss.2010.08.002>. Macey, M. C., Fox-Powell, M., Zambrano, N. K., Stephens, B. P., Olsson-Francis, K. (2020). The identification of sulfate oxidation as a potential metabolism during primary production on the Western Mars. *Scientific Reports*, 10(1), 19941. <https://doi.org/10.1038/s41598-020-21915-8>.